APR 19 1948
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VOLUME 6 NUMBER 4

NUTRITION REVIEWS

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Published Monthly by

THE NUTRITION FOUNDATION, INC.

CHRYSLER BUILDING NEW YORK, N. Y.

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NUTRITION REVIEWS

VOL. 6 APRIL, 1948 NO. 4

WE NEED MORE NUTRITIONISTS

Scientists, building upon the foundation laid by pioneers in the field of nutrition, have in recent years advanced our knowledge to the point where we now know what food we need to be free from nutritional diseases. More than that, they can prescribe the diets that will enable many of us to live healthier, happier, and longer lives. Great as their knowledge is, however, we have reason to believe that the science of nutrition holds still greater gifts of health in store for us—that we have, as yet, only skimmed the surface secrets from this new science.

The most critical issue today in foreign and domestic affairs alike, is the allocation of food. Despite the fact that we now have the knowledge which could assure every man, woman, and child in the world of the food essential to health, starvation stalks abroad: and even in rich, fertile America, malnourishment is widespread.

The reports of former President Hoover's missions make it apparent that this country must furnish food to much of the rest of the world if utter chaos is to be avoided. However, even as we accept the responsibility for nourishing the people of wartorn lands, we wonder how we can do it without depriving our own citizens of the food they need. Already soaring food prices make it impossible for many American families to maintain a healthful diet and there is fear that prices will go still higher if food exports are increased.

Is there a way out of this dilemma? Can we meet our foreign obligations and at the same time protect the health of our people at home? World peace as well as world prosperity depends upon an affirmative answer to that question.

One positive thing that can be done is to decrease our wastage of food. Unofficial estimates indicate that food wastage from all sources now runs as high as 24 per cent and that from 5 to 10 per cent of food that could be salvaged goes into the family garbage pail. Whether this estimate is too high or too low, I am not prepared to say, but there is no doubt that food wastage exists and that it should be reduced during this year of crisis.

The need to apply nutrition knowledge to attain maximum health on minimum diets is more acute now than ever before. During the war, nutrition committees formed in every state and almost every county enabled volunteers to join with professional nutritionists in a comprehensive program that brought nutrition information to millions of housewives. The professional nutritionist, as well as the layman, learned much from this wartime cooperation that they can apply to the present crisis.

Nutritionists, for example, are in a better position than most to point out that the present situation is not entirely an aftermath of war. No nation, not even America, has ever produced enough of the right kinds of food to meet the needs of adequate nutrition for its entire population. During the depression, thousands of citizens bore the marks of malnutrition. Producers of food were threatened with economic ruin and at the same time people who needed food could not afford to buy it.

The recent war further underscored for us the economic nature of the nutrition problem. At that time, due to full employment and higher wages, people could purchase the food they needed and as a result, although more food was available to civilians than ever before, there was not enough to meet the demand and rationing had to be established to alleviate the shortage. The nation's

consumption of food in wartime was phenomenal, but even then many families lived on a food supply below the level needed for optimum health. We need never fear overproduction if we can only work out adequate distribution of food to meet the health needs of everyone.

Nutritionists have a clear responsibility to make these facts better known so that plans now in the making will be geared to meet long range as well as immediate needs for expanded production. We alone cannot solve the problem of poverty which has always been a basic cause of malnutrition; but we can help to solve it by letting the people know the vital role nutrition plays in building and maintaining vigorous health. Next to poverty, ignorance is the principal reason why deficiency diseases continue to take an unnecessary toll of life and a heavy toll of health. Ignorance is the principal reason why we consume only half as many leafy green and yellow vegetables as we need and why many people, even in the goiter belt areas of the country, continue to use uniodized salt. Nor can poverty be entirely blamed for the failure of many women to give vitamin D milk to their babies, or to insist upon getting enriched bread and flour. If the public realized what a proper diet could contribute to their health, they would demand laws requiring all salt to be iodized: all white bread and white flour to be enriched.

The fact that nutritional deficiencies are found in families of all income groups and among people with the most as well as with the least general education, shows that nutritionists still have a long way to go in presenting their findings in terms that will impel people to improve their food habits.

The school lunch program is one effort to bring more knowledge of nutrition to the people. Properly handled, it accomplishes the dual purpose of supplementing inadequate home diets and of training children to want and like the foods essential for health. The assets of this program are tremendous, yet, even today, many schools are operating with totally inadequate lunching facilities and there are still people who do not realize the false economy of providing expensive buildings, equipment, and instruction to a child whose mind is dulled and whose body is stunted because of malnutrition. However, the public generally is accepting the school lunch program as a part of the school system. This is a great step forward.

Another significant advance is the expansion of the industrial lunch program. Prior to the war, many companies had no in-plant feeding facilities, or very poor ones. However, management and labor alike soon discovered during the war that giving the workers a nourishing meal not only enabled them to do more and better work, but also reduced absenteeism.

The most recent and promising effort to improve nutrition is the development of nutrition programs in public health departments. The modern health department is much more than an agency to prevent the spread of communicable diseases and to see that sanitary codes are observed. It is concerned with every aspect of preventive medicine. And, since nutrition is one of the most important aspects of preventive medicine, nutrition education must assume increasing importance in every health department program.

Recognizing that health officials are in a particularly favorable position to spread nutrition knowledge, the State and Territorial Health Officers requested the aid of the U. S. Public Health Service in setting up nutrition services. As a result of this request, the Service has established four field investigation and demonstration units, and more are needed. These units are staffed with a medical officer, a public health nurse, a nutritionist, and a biochemist. Working closely with the state and local health departments, the demonstration units aim to accomplish several things: to detect nutritional deficiencies in their early stages and help people to overcome them; to find

out how well or how poorly nourished the community is; to train local health department staffs so that they can continue the program; and to develop patterns of operation which can easily be adopted by state and local health departments that wish to start an efficient, economical nutrition service. In these and other ways we have begun to take nutrition knowledge out of the scientist's laboratory and put it to use in the kitchens of our homes, schools, and industries.

Though much is already known about the science of nutrition which is ready for application, there is still great need for further fundamental research. Within the past two years, the U. S. Public Health Service has granted over 10 million dollars to further research activities in research institutions throughout the country. Since allocation of research funds is made entirely upon the recommendation of 250 of the nation's leading scientists, the projects they select as worthy of support from tax funds afford a clue to the importance of a subject from the research standpoint. Nutrition and biochemistry rank high in terms of the amount of federal research funds alloted to their study. This is tangible evidence that nutrition research is a most promising field for student scientists to prepare to enter.

At the first United Nation's Conference on Food and Agriculture, the United States and 43 other nations formally endorsed the democratic principle that everyone is entitled to an opportunity to secure a diet adequate for health, that it is the responsibility of governments to assure this basic human right, and that agricultural policies both national and international must be directed toward this end. It was recognized that the immediate attainment of such a goal is out of the question but that progressive improvement in national food supplies is possible. If the world production and use of food can be based on freedom from want for everyone everywhere, we can have an age of health and prosperity exceeding anything ever known.

W. H. Sebrell, Jr.
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DIET AND ARTERIOSCLEROSIS

Although life insurance statistics plainly indicate that the so-called "degenerative" diseases are the prime causes of death in human beings and promise to be an increasing cause as the mean age of the population increases, there is surprisingly little evidence of interest and organized effort to study the factors which predispose to, and accelerate, the development of sclerosis of the blood vessels.

It has been known for many years that by chemical analysis the arterial plaques found in individuals who die of or with this disease contain excessive amounts of lipid materials, particularly cholesterol and its esters (A. Windaus, *Z. f. physiol. Chem.* **67,** *174 (1910)*). It is also well known that certain of the metabolic diseases associated with an elevation of the serum lipids are often associated with an unusual degree of arteriosclerosis. Thus diabetics are known to be especially prone to the development of vascular disease (H. Dolger, *J. Am. Med. Assn.* **134,** *1289 (1947)*). This continues to be true even though good regulation with diet and insulin have almost removed the presence of acidosis and abnormal lipemia. The persistence and apparent increase of arteriosclerosis in diabetics since the introduction of protamine zinc insulin which allows maintenance of apparently normal lipid metabolism gives less credence to an hypothesis which implicates abnormal lipid metabolism as a cause. Individuals with diabetes serve

not only as a challenge for a solution of this problem but also as the most useful investigative material available for study.

An early experimental approach to this problem by N. Anitschkow (*Verhandl. d. deutsch. path. Gesellsch.* **23,** *473 (1928)*) revealed that inclusion of cholesterol in the diet of certain herbivorous animals led to the deposition of cholesterol plaques in the arteries. These experiments, continued in this country especially by T. Leary (*Bull. N. Y. Acad. Med.* **17,** *887 (1941)*), have been criticized because the animals used (rabbits) were those in which no natural mechanism for the disposition of cholesterol is present. Some doubt is thus justifiable when these data are extended to include the human disease which they are meant to simulate. There is considerable evidence that the nature of the diet which is habitually consumed is an important factor in the course of vascular disease. This evidence was well summarized by S. R. Rosenthal (*Arch. Path.* **18,** *473, 660, 827 (1934)*) some years ago. It is of interest that among the lower animals atherosclerotic plaques are seen occasionally. H. Fox (see E. V. Cowdry, "Arteriosclerosis," *p. 153, The Macmillan Company, New York (1933)*) has pointed out that among birds the Psittaciformes which normally eat animal foods containing significant amounts of protein and lipids including cholesterol have a significant degree of sclerosis at autopsy. These lesions simulate the human lesions. Similarly it has been suggested that the racial differences among human beings are due to differences of habitual diet. One of the significant gross differences in the diet of oriental peoples who seem singularly free of vascular sclerosis even at advanced ages is the much smaller amount of fat in their diets. Whether it is the fat content of the diet alone or in combination with other factors that are responsible, is undetermined. If one were to continue this argument, an examination of the vessels of races which continually eat foods containing large amounts of fat would be necessary. There is a real need for more extensive studies of this type which include careful histologic surveys rather than measurement of a single clinical manifestation such as the blood pressure.

A study pertinent to this problem has been published by S. L. Wilens (*Arch. Int. Med.* **79,** *129 (1947); Am. J. Path.* **23,** *793 (1947)*). The relation of obesity to the incidence of atherosclerosis has been investigated in routine autopsy cases in a New York hospital. Although no diet histories were available, the author relates overnutrition, as measured by body weight, to the frequency and severity of aortic sclerosis. The close correlation of obesity with atherosclerosis is entirely compatible with the inferences previously drawn from the deleterious affect of obesity upon life expectancy as portrayed by vital statistics. Also of interest is the observation that the frequency of atheromata is considerably less in people who die after a wasting disease, leading the author to conclude that the aortic lesions are to some extent reversible. The yellow streaks so often seen beneath the intima of vessels in infants has generally been considered to be a reversible deposition of lipid material. The relation of the latter to the structural changes and adaptive reaction of the vessel wall as manifested in true arteriosclerosis has not been determined.

J. R. Moreton (*Science* **106,** *190 (1947)*) has published a preliminary report of a study on the effect of the physical state of the blood lipids upon the development of vascular sclerosis. The substance of this report is concerned with a method which is intended to measure the particle size of the emulsified fat in the plasma. The hypothesis which the author presents states that the formation of atherosclerotic plaques is the result of the presence of abnormally large chylomicra in the plasma of the affected individuals after a fat meal. It is supposed that the vascular damage results from embolization of these "macro-chylomicra" in the vasa vasorum. Since the chylomicra are known to contain

cholesterol, it is believed that the triglycerides and phosphatides are then easily reabsorbed leaving a deposit of cholesterol in the vessel wall. Although an interesting theory, there are as yet no significant data to support it. The method which Moreton has used for the measurement of the particle size is by no means a critical measurement and rather measures the mean particle size of the total chylomicra present. Lacking data concerning the dimensions of the vasa vasorum there is in addition no evidence to support the belief that embolization occurs.

Nutritional studies of the past twenty-five years have revealed that even in acute experiments the nature of the diet fed will affect the state of the vessels. L. M. Polvogt, E. V. McCollum, and N. Simmonds (*Bull. Johns Hopkins Hosp.* **34,** *168 (1923)*) showed that maintenance of rats on a diet containing large amounts of protein would lead to development of renal lesions. L. E. Farr and J. E. Smadel (*Proc. Soc. Exp. Biol. Med.* **36,** *472 (1937)*) have shown that the level of the diet protein will influence the course of experimental nephritis in rats. H. Selye (*Canad. Med. Assn. J.* **50,** *426 (1944)*) has shown that the diet of rats subjected to the "alarm reaction" by any of several procedures would determine the amount of vascular and renal damage which results.

In the absence of experimental data the clinician has been given but little help with the dietary management of the problems of vascular sclerosis. Low protein diets have been in vogue for treatment of nephritis but these procedures are of doubtful value and in many instances are being replaced with more liberal diets. The use of low protein, low salt diets in the treatment of hypertension is directed at the symptom rather than the fundamental disease. Low fat diets have been advocated, particularly in the attempt to prevent vascular complications in the diabetic, but these are not generally accepted and based at best upon incomplete evidence.

The nutritional approach to the major problems of vascular sclerosis and neoplastic disease may prove most successful.

FOLIC ACID IN TREATMENT OF RHEUMATOID ARTHRITIS

Requiring mention, since it may be quoted uncritically, is a study of the use of folic acid in the treatment of anemia in rheumatoid arthritis by C. A. L. Stephens, A. L. Borden, W. P. Holbrook, and D. F. Hill (*Ann. Int. Med.* **27,** *420 (1947)*). After an appraisal of previous studies of the value of various therapeutic agents in the management of the anemia associated with rheumatoid arthritis in which it is pointed out that treatment with iron, liver extract, crude or purified, and other measures have proved of little or no value, an uncritical account is given of the writers' own observations on 20 patients who were treated with folic acid. Their patients were divided into two groups of 10 each. The first group received 5 mg. of pteroylglutamic acid by mouth four times daily and were given no other treatment for their anemia. The second group received, in addition to this amount of folic acid, ferrous salts in dosages of 200 mg. of metallic iron per twenty-four hours.

What the investigators call an appreciable reticulocytosis, a rise in the mean corpuscular volume, hemoglobin, color index, and volume of packed red cells following folic acid therapy in the 10 patients receiving only folic acid was a rise in reticulocytes from an initial mean average of 1.83 per cent to a mean average of 4.22 per cent, a change in mean corpuscular volume from 69.1 to 72.5 cubic microns, a rise in hemoglobin of 0.496 g., a difference in mean average color indexes of 0.029, and a mean average increase in volume of packed red cells in two weeks of

1.87 per cent. Subsequent changes in these patients as well as in the second group were of a similar order. The comment is made that along with these changes there was an improvement in the morphology of the erythrocytes which, prior to folic acid therapy, were "sickly in appearance." A peculiar finding was the discovery of nucleated red cells in the peripheral blood and immature white cells following folic acid therapy.

Except for some of the morphologic changes to which the authors refer, the changes in the size and hemoglobin content of the red cells, in hemoglobin, and in volume of packed red cells which they record are well within the limits of expected variation. It is difficult to understand how these workers can find justification for the statement that folic acid improved the blood picture in their patients. It is of interest that no symptomatic improvement occurred in their cases.

COPPER DEFICIENCY IN RABBITS

A deficiency of copper produces anemia in several species of animals. S. E. Smith and G. H. Ellis (*Arch. Biochem.* **15,** *81* (*1947*)) have now confirmed their earlier observation that a deficiency of this element produces an anemia in the rabbit (S. E. Smith, M. Medlicott, and G. H. Ellis, *Am. J. Physiol.* **142,** *179* (*1944*)). Black and white Dutch rabbits were given access to whole milk powder in addition to their dams' milk until weaned. They were then fed a basal diet of whole milk powder ad libitum plus a daily supplement of iron, manganese, and a fish liver oil. One group received in addition 0.4 mg. of copper per rabbit per day. Two series of experiments were run, 7 animals in each group in the first series, and 18 in each group in the second. In the first series, hemoglobin levels were determined on 5 of the copper deficient animals. These were not significantly lower than the hemoglobin levels of the controls. In the second series, hemoglobin determinations were made at the end of twenty weeks on the experimental regimen. The mean values were 12.3 ± 0.62 g. per 100 ml. for the controls and 10.1 ± 0.65 g. per 100 ml. for the deficient group of 13 animals. These differences in means are statistically significant (5 times in 100).

The copper deficient animals grew somewhat less than those receiving copper. A large percentage of the deficient animals developed achromotrichia (graying of the hair), alopecia (loss of hair), and, a smaller percentage, a dermatosis characterized by dry flaky noninflammatory encrustations. That copper deficiency can result in depigmentation may possibly be related to the fact that tyrosinase, an enzyme which acts on tyrosine (a probable precursor of melanin), is a copper-containing protein.

A PROTECTIVE EFFECT OF STARCH IN CHOLINE DEFICIENCY

Various relations of dietary components to the hemorrhagic kidney syndrome of choline deficiency have been reviewed (*Nutrition Reviews* **4,** *345* (*1946*)). W. H. Griffith (*J. Nutrition* **22,** *239* (*1941*)) stated that starch prevented the development of hemorrhagic kidneys in young white rats under the conditions of a choline deficient regimen. On the other hand, P. Handler and I. N. Dubin (*Ibid.* **31,** *141* (*1946*)) and P. Handler (*Ibid.* **31,** *621* (*1946*)) did not observe any appreciable difference when starch was substituted for sucrose as the dietary carbohydrate. In more extensive studies J. H. Baxter (*J. Nutrition* **34,** *333* (*1947*)) demonstrated that when the carbo-

hydrate component of his diets was corn starch, there was an appreciably lower incidence in the signs of renal insufficiency than when sucrose was used as the carbohydrate. For example, 16 per cent of the 44 white rats fed the corn starch diet died with hemorrhagic kidneys, while 62 per cent of the 50 white rats fed the sucrose diet died with evidence of kidney hemorrhage. When the corn starch was assayed by the reineckate method, it was found to contain 13 to 15 mg. of choline per 100 g. Therefore the amount of corn starch in the diet contributed approximately 80 mg. of choline per kilogram of diet. The addition of 100 mg. of choline chloride to 1 kg. of the sucrose ration did not decrease the incidence of hemorrhagic kidneys but was sufficient to increase the rate of growth of the rats and to delay the onset of hemorrhagic kidneys by one or two days. A supplement of 200 mg. of choline chloride per kilogram of sucrose ration was necessary to give the same degree of protection with respect to the hemorrhagic kidney syndrome as the substitution of corn starch for sucrose. J. P. Fletcher, C. H. Best, and O. M. Solandt (*Biochem. J.* **29,** *2278 (1935)*) stated that the choline content of corn starch as determined by acetylation and bioassay of the resulting acetylcholine on the isolated intestine of the rabbit was 25 mg. per 100 g. Even if this higher value is correct, the amount of choline introduced into the ration by the starch in Baxter's experiments would probably still be insufficient to result in the reduction observed in the above experiments. Since the food intake of the rats on the starch diet was greater than on the sucrose diet, the effect of starch could not be attributed to a reduced caloric intake which has been demonstrated to result in a reduced incidence of hemorrhagic lesions (*Nutrition Reviews* **4,** *345 (1946)*).

In an effort to determine if other factors in the corn starch might be involved, samples were treated by shaking twice with distilled water and by extracting three times with methyl alcohol and once with ether. The extracted starch and the extracts were each evaporated to dryness. The combined residues from 1500 g. samples of starch were added to 500 g. of sucrose ration. If extraction of the starch had been complete and no destruction had occurred, the resulting diet would contain the extracted materials in about five times their concentration in the starch diet. The animals maintained on the sucrose rations supplemented with extract residues did not have an appreciably different incidence of hemorrhagic kidneys than the rats on the unsupplemented sucrose ration. Choline determinations on the extracts indicated that only one third of the choline had been extracted. Thus the extract residues contributed about 140 mg. of choline per kilogram of ration. To test whether the active principle in the starch had been destroyed in this mild extraction process, the extracted starch was used to replace sucrose in the diet. The resulting low incidence of hemorrhagic kidneys occurring in the rats maintained on the extracted starch diet was comparable with that observed in rats fed the diet with unextracted corn starch. Thus the protective effect of starch appeared to be due to the starch *per se* or to some material not extracted.

The addition of 2 per cent of succinylsulfathiazole to the starch diet did not result in any difference in the incidence of hemorrhagic kidneys. The cecums of the animals receiving succinylsulfathiazole were much larger at the end of the experimental period than in normal rats. This enlargement was not related to the choline deficiency since it also occurred in rats which had received 0.2 per cent choline chloride in addition to the succinylsulfathiazole supplement. The accompanying alterations in the intestinal flora neither interfered with nor enhanced the protective effect of the corn starch. Analyses failed to demonstrate the presence of appreciable amounts of choline in the feces from various groups of choline

deficient rats. However, previous observations have indicated a lower incidence of hemorrhagic kidneys when animals were caged in groups instead of in individual cages (*Nutrition Reviews* **4,** *345 (1946)*) which suggests the intestinal synthesis of choline. It is possible that such an effect might be demonstrated if other "sulfa" compounds were used. Biologic assays of the ability of the feces from choline deficient rats to prevent hemorrhagic kidneys in rats maintained on choline deficient rations would be valuable as a check on the chemical determination for choline. On the other hand, the presence of an unrecognized source of labile methyl within the starch granule has not been ruled out. The observations of T. C. Taylor and J. M. Nelson (*J. Am. Chem. Soc.* **42,** *1726 (1920)*) are instructive in this respect and emphasize the possibility of error in the analysis of corn starch. Successive thirty-six hour extractions with ether, petroleum ether, and carbon tetrachloride yielded only 0.1 per cent of lipids but a subsequent extraction of an acid hydrolysate of this "fat-free" sample showed the presence of an additional 0.6 per cent. It may be that compounds containing labile methyl are similarly masked in the intact starch granule.

VACCENIC ACID IN NUTRITION

Vaccenic acid is an unsaturated fatty acid that is also known as iso-oleic acid. It has the same structure as oleic acid except that the double bond is in a different position. J. Boer, B. C. P. Jansen, and A. Kentie (*Nature* **158,** *201 (1946); Nutrition Reviews* **5,** *96 (1947)*) claim that this material is an essential growth promoting factor and it was obtained by them from "summer butter."

The details of the study of the essential nature of vaccenic acid for the rat have been presented by these workers (*J. Nutrition* **33,** *339 (1947)*) who also postulated that the unsaponifiable fraction of "summer butter" has a growth promoting action in addition to that of vitamins A and D. Evidence for the requirement of the rat for vaccenic acid was based on the reduction of growth promoting activity of "summer butter" after hydrogenation and of the filtrate of summer butter after treatment with fuller's earth. Fractionation and separation yielded samples of fatty acids high in vaccenic acid which were reported to give a growth stimulus comparable to that of summer butter when fed to rats on diets with the fat supplied by olive oil or rapeseed oil. A further test of vaccenic acid in this capacity was made by J. Boer, B. C. P. Jansen, A. Kentie, and H. W. Knol (*Ibid.* **33,** *359 (1947)*) who prepared by hydrogenation, a fraction high in vaccenic acid from Chinawood oil, a fat consisting largely of alpha-oleostearic acid. Again a growth promoting action was reported. R. P. Geyer and co-workers (*J. Biol. Chem.* **169,** *227 (1947)*) analyzed various animal and plant fats for vaccenic acid. Butterfat contained the most, while plant oils contained none by the method used. If this method can be accepted as sufficiently sensitive, the fact that no vaccenic acid was found in plant oils would suggest that the requirement of the white rat for some dietary fat in addition to the essential unsaturated fatty acids could not be satisfied by vaccenic acid and that there must be some third entity of nutritional importance. The various controversies relative to the possible superiority of butterfat over vegetable fats have been discussed previously (*Nutrition Reviews* **2,** *267 (1944)*; **4,** *140 (1946)*).

Recent investigations from the laboratories of H. J. Deuel (*Nutrition Reviews* **6,** *80 (1948)*) and the studies from Jansen's laboratory do not answer many of the questions about this interesting problem.

TOOTH DECAY IN THE COTTON RAT

A number of interesting results relating dietary intake to tooth decay in the cotton rat have been previously discussed in this journal (*Nutrition Reviews* **3,** *83 (1945);* **4,** *73, 308 (1946)*). The principal findings may be summarized as follows: (a) a high incidence of carious lesions is observed in the molars of cotton rats fed a dry, synthetic-type ration high in sucrose or other fermentable sugar; (b) the incidence of caries can be considerably lowered by increasing the fluorine intake, by replacing the fermentable carbohydrate of the ration by a less fermentable carbohydrate such as dextrin, or by feeding certain natural foodstuffs; (c) animals fed a diet consisting entirely of liquid whole milk are protected completely from tooth decay; (d) a solid diet approximating the composition of milk solids is less effective in protecting against tooth decay than is liquid whole milk, but is far more effective than dry rations containing sucrose; (e) isocaloric substitution of fat or of protein for part of the sucrose in a dry ration decreases the incidence of dental caries.

In a new series of experiments using the same general procedures of litter mate distribution, and evaluation of the incidence and extent of carious lesions, E. P. Anderson, J. K. Smith, C. A. Elvehjem, and P. H. Phillips (*Proc. Soc. Exp. Biol. Med.* **66,** *67 (1947)*) confirm previous observations showing that a diet of liquid whole milk fortified with iron, copper, manganese, and liver extract completely prevents development of carious lesions in this animal. They then describe the effects of adding sucrose, glucose, or Dextri-maltose to such a liquid milk diet. When any one of these fermentable carbohydrates is used as the sole carbohydrate in a dry ration, the resulting ration is highly cariogenic. A dry, sucrose-containing ration was fed as a control. The incidence of carious lesions after fourteen weeks on this control ration averaged about 30, as compared with 0 for the group fed mineralized milk. The effects of adding the above carbohydrates to the milk ration on the incidence of dental caries was as follows: 5 per cent of sucrose, no effect; 10 per cent of sucrose, average number of lesions raised to 4; 5 or 10 per cent of Dextri-maltose, no effect (incidence 1 and 0, respectively); 10 per cent of glucose, average number of lesions raised to 6. Thus a high degree of protection against dental caries is afforded by the liquid milk diet even when large amounts of fermentable carbohydrates are incorporated into this ration. It is interesting to note that the cotton rats which received additions of 10 per cent of sucrose, Dextri-maltose, or glucose to the milk were obtaining slightly in excess of one third of their caloric requirements from the added carbohydrate.

In a further experiment, the cariogenic control ration was fed together with milk ad libitum. Approximately one third of the caloric requirement of the cotton rats was supplied by milk. The incidence of carious lesions was reduced from 31 to 14 by the milk supplement, and the extent of the lesions in the latter group was much less than in the former. These results again indicate the marked protective effect of milk, even when fed as a supplement to a dry caries-producing ration.

The mechanism by which milk exerts its effect is unknown. The physical state of the ration may play a role. In the liquid diets, carbohydrates are dissolved, and probably remain in the mouth a comparatively short time, whereas in mastication of a dry ration the teeth would be in contact with fermentable carbohydrates for much longer periods. Animals on the milk diets grew somewhat more slowly than those on the control synthetic-type ration; a difference in caloric intake may thus also have been involved. Further work will be neces-

sary to evaluate the role played by each of these various factors.

While considering the possible role of liquid rations in their relation to tooth decay, it is interesting to consider at the same time a condition which accentuates rather than reduces the retention of food debris in the oral cavity. J. E. Gilda and P. H. Keyes (*Proc. Soc. Exp. Biol. Med.* **66,** *28 (1947)*) desalivated small groups of male and female hamsters. A slight but not significant increase was observed in the dental caries scores of male hamsters; a large, significant increase was observed in female hamsters. Since the groups were small, the increase observed among males might have been significant if the same degree of increase had been obtained in a large group of animals. One possible explanation of the higher percentage increase among females may be centered around the much lower caries susceptibility of normal female hamsters than that of normal male hamsters. Desalivation has already been shown to result in a much higher occurrence of tooth decay in white rats fed a coarse corn meal ration (D. Weisberger, C. T. Nelson, and P. E. Boyle, *Am. J. Orth. Oral Surg.* **26,** *88 (1940)*). Agenesis, greatly reduced function, or extirpation of salivary glands in man have also been demonstrated to result in an increased rate of tooth decay (H. Prinz, *Dental Cosmos* **74,** *129 (1932)*; M. Faber, *Acta Paediat.* **30,** *148 (1942)*). In this particular instance, the data obtained with two species of rodents parallels that in man. This is of special interest in this case since the type of carious lesion in the hamster originates primarily on the smooth surfaces of the teeth while those in the white rat originate largely from mechanical injury of the tooth surfaces by the coarse corn meal particles.

SYNTHETIC STEROLS AND THE GUINEA PIG ANTISTIFFNESS FACTOR

The antistiffness factor for guinea pigs discovered by A. M. Bahrs and R. Wulzen (*Proc. Soc. Exp. Biol. Med.* **33,** *528 (1936)*; *Physiol. Zool.* **9,** *508 (1936)*) has been studied in considerable detail by W. J. van Wagtendonk and co-workers. Two previous reviews (*Nutrition Reviews* **1,** *292 (1943)*; **5,** *76 (1947)*) have discussed certain phases of their work, which culminated in the isolation from sugar cane juice of a pure white crystalline compound, melting point 81.5° to 82°C., which reportedly cured wrist stiffness in guinea pigs when fed in five daily doses of 0.002 microgram each. These reviews emphasize the need for confirmation of the work by other laboratories, as well as for information regarding the reliability and limitations of the assays. Experiments of this nature have been published by J. J. Oleson and coworkers at the Lederle laboratories (*J. Biol. Chem.* **171,** *1 (1947)*) which permit an evaluation of the earlier work.

The diet used by the Lederle workers consisted of ground wheat 20 pounds, ground corn 15, oatmeal 20, wheat bran 5, soybean meal 20, linseed oil meal 5, fish meal 2, alfalfa leaf meal 10, bone meal (steamed) 1, limestone 1, sodium chloride 4 ounces, delsterol 2 ounces, and ascorbic acid 12 g. Each guinea pig received an additional 20 mg. of ascorbic acid orally three times a week. On this diet (fed in the form of pellets) animals developed deficiency symptoms which were considered to be identical to those produced by R. Wulzen and A. M. Bahrs (*Am. J. Physiol.* **133,** *P500 (1941)*) on a skim milk diet. The stiffness could, in turn, be cured by 1 to 5 micrograms of van Wagtendonk's crystalline antistiffness factor. Assays were carried out using the 1 to 4 plus system described by W. J. van Wagtendonk and R. Wulzen (*J. Biol. Chem.* **164,** *597 (1946)*).

The Lederle group found over twenty-four known steroid materials to possess antistiff-

ness activity. Several of the most active compounds were esters of ergostanol, which, along with ergostanol itself, gave responses in doses as low as 1 to 5 micrograms. Apparently the ring saturation present in ergostanol is important for activity, since compounds in this series with less degree of saturation were less effective.

Oleson and co-workers have thus confirmed the existence of the stiffness syndrome, as well as the curative power of the material derived from cane juice. However, they do not confirm the isolation of a factor active in 0.002 microgram dosage. This five hundred fold discrepancy seems to be multiplied further by a report by D. H. Simonsen and W. J. van Wagtendonk (*J. Biol. Chem.* **170**, *239* (*1947*)) that the activity of the crystalline factor is actually due to the presence of traces of an impurity melting at 164°C. Judging from this statement, as well as from experience with melting point curves of other mixtures, one might conclude that the impurity could not be present in a concentration greatly exceeding 1 per cent of the mixture. The activity of the high melting component would thus be of the order of 0.00002 microgram—a vanishingly small figure.

Further examination of the Lederle data reveals that the stiffness test, in their hands at least, is altogether inadequate for purposes of isolation, for three reasons. First, when the deficient animals were given test doses to measure the curative power of various substances, the negative controls (animals receiving no active material) were frequently benefited as well as those receiving treatment. Thirteen per cent (15 of 113 animals) showed marked improvement, and an additional 17 per cent showed slight improvement. Second, tests of the antistiffness factor over a fivefold range (1 to 5 micrograms) and of ergostanyl acetate over a tenfold range (5 to 50 micrograms) indicated activity in each test, but no change in the per cent of cures over these concentration ranges. In other words, it would be impossible to detect a separation of active material into two fractions, even when a 10:1 distribution ratio is obtained. To obtain data of quantitative significance, the range would seemingly have to be extended at least an additional tenfold. Although ergostanyl acetate gave complete cures at the 100 microgram level, tests of this compound over a fiftyfold range (1 to 50 micrograms) provided a variation in per cent positive responses ranging from 45 to 67—barely outside the limit of reliability of the test as determined with negative controls. Third, eleven esters of ergostanol were active. However, wide variations in activity were noted among the compounds in a homologous series; for example, the isovalerate gave 64 per cent positive responses, the isocaproate none; the laurate, palmitate, and stearate gave 38, 67, and 0 per cent, respectively. It is difficult to attach much significance to these values, especially since in any case the ester probably is of value only for its ergostanol content.

It is possible that the assay method has proved more reliable in the experience of van Wagtendonk's group. This is implied by their expression of results to two significant figures, and the postulation of an antagonistic factor, adsorbable on magnesium oxide, the removal of which resulted in a threefold increase in total activity (*J. Biol. Chem.* **164**, *597* (*1946*)). However, if this is true, other observations seem doubtful: one, pointed out in a previous review, is the over-all yield of 67 per cent of the active material from cream after six different operations, meanwhile effecting a six million fold concentration (*Arch. Biochem.* **1**, *373* (*1943*)). An over-all yield of 83 per cent was later reported (*J. Biol. Chem., Ibid.*) after fourteen operations with cane juice. Another (*Arch. Biochem., Ibid.*) is the conversion of the pure mercury complex to the free ketone (steps F and G) wherein roughly 85 per cent of the pure material was lost without any corresponding change in total activity. On the other hand, if, as seems probable, the assay

method was not quantitative, the various steps in the concentrations could hardly have been followed. The treatments given the cream and cane juice could be expected to concentrate steroid material, and since activity seems to be shared by a number of compounds it is possible that pure fractions isolated in the manner described would be found active, the assay notwithstanding.

For the present, it appears that further work is necessary to establish the isolation of the pure factor from natural sources. Once established, a comparison of structure and activity with those of synthetic sterols would be of especial interest, as well as an examination of possible beneficial effects of these compounds in human nutrition and metabolism. Finally, the observations by van Wagtendonk that deficient animals suffer an upset in calcium and phosphate metabolism raise the question whether the stiffness syndrome represents a true nutritional deficiency or a pathologic condition which may be brought about by excesses of, or improper balances between calcium and phosphorus in the special diets employed. Elucidation of this point would be of importance in evaluating the physiologic significance of this interesting syndrome.

RELATION OF PROTEIN DEFICIENCY TO EXPERIMENTAL WOUND HEALING

While it is generally admitted that protein is important in the healing process, the manner in which lack of protein affects healing is not clear. To obtain objective information on this subject, M. W. Kobak, E. P. Benditt, R. W. Wissler, and C. H. Steffee (*Surg. Gyn. Obstet.* **85**, *751 (1947)*) carried out some experimental studies in rats.

A tensile strength apparatus was developed for the study. By means of slow release of air from a reservoir bottle into an intra-abdominally placed rubber bag it was possible to measure with a manometer the amount of pressure necessary to disrupt wounds of the abdominal wall of rats. This measurement was recorded automatically with a kymograph. After the point of separation had been determined, the pressure necessary to expand the rubber tube through the previously disrupted incision was also established. The latter figure was considered the base line and represented the pressure dissipated in expansion of the balloon against the enclosing tissues of the peritoneal cavity and through the wound itself. By subtracting the point of separation from the base line an objective estimate of the tensile strength of the wound could be obtained.

Young, adult, male albino rats weighing initially from 180 to 220 g. were used in these experiments. These were divided into two groups, one of which was maintained on a low protein ration containing 1.9 per cent protein and the other on a control ration containing 21.1 per cent protein. These diets were isocaloric and contained an adequate quantity of vitamins, minerals, and fat. Food was offered in weighed quantities daily. The protein depletion period was three months. Normal control animals eating practically all of the diet offered consumed between 1150 and 1250 calories per square meter of body surface per day. The smaller, protein depleted animals consumed between 1350 and 1850 calories per square meter per day.

Following the period of protein depletion the animals were fasted for approximately eight hours, weighed, and anesthetized. A wound was then produced by an upper abdominal left paramedian incision of approximately 2.5 cm. in length. This was repaired with stainless steel wire sutures. On selected postoperative days the animals were again fasted, weighed, and anesthetized with ether, and blood was withdrawn for serum protein determination. The rats

were killed, the sutures gently removed, and a small incision placed in the midline beneath the wound. The catheter with rubber bag was inserted high in the peritoneal cavity. Tensile strength determinations were made on the first, second, fifth, and seventh days in both animal groups. In addition, to determine the influence of osmotic factors on the healing process, a filtered, autoclaved solution of 6 per cent gum acacia was given daily via the tail vein to control and hypoproteinemic animals and tensile strength determinations were made.

During the first two postoperative days there was little difference between the tensile strength of wounds in either dietary group. However, on the third day a divergence occurred in the protein depleted animals with a plateau in the tensile strength curve maintained up to the fifth postoperative day. At this time the wounds of the normal animals possessed a strength approximately three times that observed in the protein deficient group. Subsequently, the healing process proceeded at an almost parallel rate, the healing in the protein deficient animals lagging by a period of approximately two days. Adjustment of the plasma oncotic pressure by means of gum acacia appeared to have no effect on the tensile strength of the wounds in either depleted or normal animals.

Histologic examinations revealed that during the first two postoperative days the tensile strength was roughly proportional to the amount and organization of the fibrin laid down at the line of incision with negligible difference between the protein depleted and control specimens. On the third postoperative day definite fibroplasia was observed with considerable deposition of reticulum. On the fifth postoperative day it was seen that the fibroblastic number and activity, with collagen formation and only moderate amounts of reticulum, were more pronounced in those animals fed the control ration. In contrast, in the protein depleted group fibroblastic activity was obviously depressed. In the latter group, reticulum was present to a greater extent along the incision and cellular response had neither the organization along lines of stress, nor the density, nor the general maturity noted in the normal animals. Tissue edema was absent and wound infection was not common although, in the protein deficient rats, there was evidence of poorer resistance with occasional instances of abscess formation along the suture channels and occasional diffuse leucocytic infiltration.

In general, it was not possible to correlate serum protein levels or percentage weight loss with wound tensile strengths. A protein depleted rat which had lost 20 per cent of its body weight exhibited as poor healing as its fellow which had lost 40 per cent of its weight. Likewise, rats with serum proteins as low as 3.2 g. per cent produced wounds with as great strength as those of similar animals with serum proteins of 5.0 g. per cent. This corroborates the observations of J. E. Rhoads, M. T. Fliegelman, and L. M. Panzer (*J. Am. Med. Assn.* **118**, *21 (1942)*) that fibroplasia occurs despite lowered serum proteins. Kobak and associates found, however, that although fibroplasia occurred in animals maintained on a low protein diet, it was marked by a delay in its early phases. These observations thus suggest that the lowered tensile strength of wounds in protein deficient rats is due to diminution in the number of fibroblasts, a decrease in their rate of maturation, and a general failure of these cells to organize in adequate density along the lines of stress. There also appears to be delay in maturation of the reticulum into mature collagen.

PATHOLOGY IN EXPERIMENTAL THIAMINE DEFICIENCY

A method for the production of what appeared to be uncomplicated thiamine deficiency in the rhesus monkey by the use of purified rations was reported by H. A. Waisman and K. B. McCall (*Nutrition Reviews* **3**, *21* (*1945*)). The pathologic findings in rhesus monkeys subjected to one or more periods of acute thiamine depletion by the above procedure have been described by J. F. Rinehart, L. D. Greenberg, and M. Friedman (*Am. J. Path.* **23**, *879* (*1947*)). The most striking lesions were observed in the nuclear structures of the central nervous system. These lesions were observed in all depleted animals but were more extensive and severe in those subjected to two or more periods of thiamine depletion. Of the 7 animals studied, bilateral symmetrical foci of degeneration were observed most commonly in the putamen, caudate nucleus, inferior colliculi, and the cerebellar vermix. Six of the 7 monkeys showed involvement of one or more of the 3rd, 6th, 8th, or 10th dorsal cranial nerve nuclei. The earliest change observed in the production of these foci of degeneration was a localized edema with separation of glia and nerve fibers and a fragmentation and disintegration of the myelin sheaths. A process similar to acute ischemic necrosis was observed in some foci. With the progression of either type of lesion, there was observed a disintegration of the axons, a degeneration of glia, and an accumulation of microglia and fewer astrocytes. Structural changes could not be demonstrated in the peripheral nerves and no significant lesions could be demonstrated in the spinal cord. The vascular proliferative reactions commonly described in Wernicke's disease were occasionally seen but were not as prominent.

Waisman and McCall (*loc. cit.*) made electrocardiogram records of some of their thiamine deficient monkeys. The heart rate of the monkeys was definitely lower than normal. The height of the R wave decreased markedly and in several of the deficient animals there was an inversion of the T wave in all three leads. Rinehart *et al.* observed that the right side of the heart in thiamine deficient monkeys was dilated, at times appearing as if the muscle were stretched. Histologic examination revealed small foci of myocardial necrosis as previously observed in experimental thiamine deficiency in pigeons, rats, and pigs (*Nutrition Reviews* **1**, *28* (*1942*); **3**, *21* (*1945*)). Another lesion was a well defined hydropic degeneration of myocardial fibers with hyperplastic nuclear changes involving in particular the subendocardial fibers presumably of the conduction system. This lesion is similar to that described by K. F. Wenckebach in human thiamine deficiency "(Das Beriberi-Herz," *Julius Springer, Berlin* (*1934*)). Various criteria for the diagnosis of the cardiac form of beriberi have been described (*Nutrition Reviews* **4**, *24* (*1946*)).

HUNGER EDEMA

The demonstration that experimental subjects maintained on a famine type of diet, deficient in calories and somewhat low in protein, developed considerable edema and retention of extracellular water without a striking reduction in the plasma protein concentration (*Nutrition Reviews* **4**, *317* (*1946*); A. Keys, O. Mickelsen, and A. Henschel, *Ibid.* **5**, *31* (*1947*)) has called into question the simple hypoproteinemia theory of famine edema. Careful field observations on patients with epidemic hunger edema are of interest as they may aid in understanding the mechanism of formation of edema and

hypoproteinemia as encountered spontaneously in famine areas. F. A. Denz (*Quart. J. Med.* **16**, *1* (*1947*)) has provided one such study. This investigation appears to have been made without the advantage of the earlier report of A. Keys, H. L. Taylor, O. Mickelsen, and A. Henschel (*Science* **103**, *669* (*1946*)) inasmuch as there is no reference in the bibliography to the observations of the Minnesota group. It serves, therefore, as an independent collaboration of many of the viewpoints of this latter group of investigators.

The edema cases investigated by Denz were from among German civilian internees. The subjects were observed as to clinical evidences of edema which were graded according to the extent as slight, moderate, or severe. In addition, total serum proteins and plasma albumin levels were determined under various conditions and on some patients determinations were made of plasma volume, extracellular fluid, plasma chloride, and urinary chlorides.

The estimations of plasma volume were carried out by a technic employing Evans' blue. The worker recognized the limitations of this technic. It was pointed out that the Evans' blue dye is adsorbed on the plasma proteins and that since edema fluids may have a considerable protein content the measurements by this technic are likely to error on the high side if leakage of the dye occurs from the vascular system into the edema fluid. Extracellular fluid was estimated by a thiocyanate technic.

Using a specific gravity method, total plasma proteins were determined on 11 healthy British subjects, on 14 internees with no history of edema, and on 20 internees with various grades of edema. The subjects with clinical edema exhibited significantly lower mean plasma proteins than the normal British subjects. However, the individuals with edema did not have statistically significantly lower plasma protein concentrations than did the internees with no edema. The absence of statistical significance may have been due to the small number of individuals studied. There was no significant correlation between the degree of edema and the serum protein levels. Some subjects with definite edema had serum protein levels as high as 6.0 g. per cent, well above the widely accepted "critical level" of approximately 5.0 g. per 100 ml. None of the internees without edema, however, had serum protein concentrations of less than 5.1 g. per cent.

Albumin, to which is attributed the predominant colloid osmotic effect of the plasma, ranged from 2.58 to 3.81 g. per 100 ml. Again, in individual cases the degree of edema was dissociated from the albumin level.

The estimations of plasma volume by Evans' blue indicated that in edematous subjects they were definitely elevated above normal—volumes as high as 3.30 liters per square meter were encountered in contrast to the accepted normal range of 1.40 to 2.50 liters per square meter. The high values were found in subjects studied within a few hours after admission to the hospital. Normal values occurred in subjects studied several days after admission. These changes in plasma volume were more rapid than was expected to result from protein regeneration. Hence, the effect of simple bed rest was investigated. Three edematous subjects were placed on bed rest alone for a five day period. There occurred increases from 6 to 17 per cent in their plasma protein concentration, from 9 to 25 per cent in their hemoglobin concentration, and a decrease in plasma volume from 15 to 34 per cent of the level on admission.

These effects on hydremia were paralleled by rapid clinical changes attributable to rest alone. The latter included: loss of edema within two to four days accompanied by a loss of 3 to 7 kg. in weight; a diuresis during the first forty-eight hours, amounting to as much as 10 liters of urine; a considerable loss in urinary chloride, as much as a 45 g. loss of chloride in a two day period;

and a decrease in plasma chloride. All of these changes were observed before the protein deficiency could have been relieved by the increased dietary intake on the hospital regimen. The diet consisted of 3000 calories including 100 g. of protein. Denz notes that these changes may be observed on rest alone with no improvement in diet.

The effects of alternate periods of rest and exercise were then investigated. Four patients with edema and previously successfully treated at bed rest with the hospital diet were changed to "an active, ambulant life." This consisted of walking about or sitting from 6 a.m. until 8 p.m. with one hour of rest at midday. Two periods of active exercise, walking for two to three hours daily, were included. The diet was unaltered. Within six days all 4 cases exhibited pitting edema of the feet and 3 showed weight gains (fluid retention). Upon return to bed the edema cleared rapidly. In 2 other patients the periods were reversed by maintaining them in a state of activity for the first seven days, then treating them by rest in bed. Throughout, these 2 patients received the 3000 calory diet. Edema continued unchanged with no significant alterations in weight, plasma proteins, or plasma volume until the patients took to bed. These studies demonstrate that exercise results in persistence of edema and that the edema disappears upon bed rest. Additional evidence of this relation between fluid retention and exercise was afforded by data on the thiocyanate space. In one case, the extracellular fluid so measured increased after a period of exercise and decreased after eight days of rest. Denz suggested that further application of this method to nonedematous cases with low plasma proteins might be enlightening.

The urinary changes in edema were reported in considerable detail. Volumes of urine as high as 5 liters per day were passed during the first two days at rest—a volume greatly in excess of intake. Nocturnal frequency was pronounced and the night volume at least equaled the day volume. This diuresis was shown to be accompanied by a great loss of urinary chloride—up to 28 g. of chloride per day. On the third day of rest a marked fall in plasma chlorides occurred and the diuresis and urinary loss of chlorides checked. Simultaneous studies of urinary and plasma chloride led to the conclusion that during rest (and diuresis) the chloride concentration of the urine was 1.4 to 1.8 times that of the plasma. During exercise, this ratio decreased to less than 1.0, indicating that when edema was developing chloride resorption by the tubules was increased, resulting in chloride retention. A reversal of this ratio took place during a second period of rest. Since the salt deficient subject is less able to excrete ingested water (R. A. McCance and E. M. Widdowson, *J. Physiol.* **91**, *222 (1937)*), Denz notes that excess chloride appears to be necessary for the excretion of edema fluid.

Studies such as these add greatly to our understanding of nutritional diseases as they are encountered clinically and to our knowledge of the many factors which influence their course.

VITAMIN A DEFICIENCY IN CATTLE

Vitamin A is an essential nutrient in cattle and the effects of a lack of this nutrient have been observed under both natural and experimental conditions. G. H. Hart and H. R. Guilbert (*Calif. Agr. Exp. Sta. Bull.* **560** *(1933)*) described the natural occurrence of avitaminosis-A in cattle grazing on ranges on which the grass was nearly completely devoid of carotene after an extended drought. These investigators

found that the daily minimum requirement of carotene for cattle weighing 130 to 500 kg. is 26 to 33 micrograms per kilogram (*J. Nutrition* **10**, *409* (*1935*)). More than this amount is necessary for reproduction and for optimal levels in milk. Vitamin A deficiency results not only in xerophthalmia and blindness but also in a number of additional abnormalities including poor reproduction, excessive discharges from the mouth and nose, swelling of joints, and convulsions. The importance of an adequate intake of the vitamin in beef and dairy cattle is obvious. In practice, prevention of the deficiency is not as simple as might be expected from the wide distribution of carotenes in plant foods. Records of the Meat Inspection Division of the Bureau of Animal Husbandry, United States Department of Agriculture, show that 651 beef carcasses were condemned between July, 1941, and December, 1946, because of anasarca or edema of the musculature and of subcutaneous tissues. This problem has been investigated by L. L. Madsen and I. P. Earle (*J. Nutrition* **34**, *603* (*1947*)), who confirmed previous reports on the relation of vitamin A deficiency to the development of edema, and there appears little doubt that the appearance of anasarca is a relatively constant deficiency sign in cattle.

The circumstances under which avitaminosis-A develops are illustrated by observations made on 41 yearling Hereford steers which were being fattened for the market on a farm in the Corn Belt. From November, 1940, to June, 1941, the ration consisted of corn and fodder, supplemented daily with 1 pound of a mixture of molasses and alfalfa. During the summer months, the animals, which were not on pasture, lost their appetite but did not show evidence of edema. Appetite was restored by feeding alfalfa hay. In September, oat straw was substituted for the alfalfa. By December, 30 of the steers had swollen legs and enlarged briskets, and one of them could hardly walk. Replacement of the oat straw with alfalfa brought about a marked improvement in two weeks and nearly complete recovery in seven weeks.

Madsen and Earle were able to duplicate this incident, experimentally, with diets either deficient or low in carotene. In the latter ration, oat straw was used as roughage and was supplemented with a grain mixture having the following composition: cracked yellow corn 80; dried beet pulp 8.5; linseed meal 10; steamed bone meal 1; and salt 0.5. The sequence of deficiency signs on this diet is illustrated by the data obtained on Steer 411, a purebred Shorthorn approximately 7 months old and weighing 613 pounds. After remaining in good health and growing well for five months, convulsions occurred, the hair coat became rough, and slobbering and edema appeared. Diarrhea began after eight months and blindness was evident after nine months. The edema and convulsions became more severe and the animal died after eleven months on the diet. In other animals, after the appearance of anasarca, supplementation of the diet with alfalfa leaf meal, carotene concentrate, crystalline carotene, cod liver oil, or distilled vitamin A concentrate in natural ester form cured the edema and all other symptoms except permanent blindness.

Blood analyses were made on 7 animals during the edematous phase and after the recovery resulting from supplementation with one of the sources of vitamin A mentioned above. The deficient animals showed low levels of plasma, vitamin A, carotene, ascorbic acid, and phosphatase. Serum calcium, magnesium, and inorganic phosphorus were slightly decreased but the values were within normal ranges. Serum albumin was decreased but fibrinogen and total globulin were increased. The total serum protein was slightly increased. The authors did not believe that the changes in blood proteins and the resulting alterations in the colloid osmotic pressure of the plasma

were sufficient to account for the severity of the edema.

Anasarca in cattle has been commonly ascribed to feeding old corn. However, in these experiments as well as in the studies of C. C. Hastings (*J. Am. Vet. Med. Assn.* **104,** *159* (*1944*)), new corn was used. It is apparent that yellow corn cannot be depended upon to supply sufficient carotene. Actually, it contains no more and often less carotene than low grade hay. The extent of conversion of carotene into vitamin A varies with the species (*Nutrition Reviews* **4,** *67* (*1946*)) and it appears that the process is relatively inefficient in cattle. Steer 411 consumed enough yellow corn at the height of the deficiency to provide 13.6 mg. of crude carotenes daily. According to the data of Guilbert and Hart (*J. Nutrition, loc. cit.*), its daily requirement was only 3 mg. of vitamin A yet this was not obtained from four times as much carotene.

Vitamin A deficiency is most unlikely in cattle on green pasture. Under such conditions, Guilbert and Hart have estimated that more carotene is ingested in a few days than is stored as carotene and vitamin A in a lifetime. However, there is marked loss of carotene in improperly cured hay (*Nutrition Reviews* **5,** *223* (*1947*)) and vitamin A deficiency is possible in cattle fed poor hay, even though yellow corn is provided as a supplement. These observations are a pertinent illustration of the difficulties which may arise through failure to recognize and to compensate for losses of nutrients in the processing of foodstuffs. They also emphasize that the mere presence of vitamin-containing foods in the diet does not guarantee an adequate daily supply. In this instance, the development of a vitamin deficiency is particularly significant because the animals were not on a restricted caloric intake but were intended to be on a fattening regimen. Edema has not been noted as a general symptom of vitamin A deficiency and its occurrence in cattle merits further study.

FOLIC ACID IN SPRUE

The hope that folic acid might be the long sought for specific chemical remedy for pernicious anemia has been somewhat dimmed by recent reports that lesions of the spinal cord develop in patients with pernicious anemia treated only with folic acid (B. E. Hall, C. H. Watkins, and M. M. Hargraves, *Proc. Central Soc. Clin. Res.* **19,** *55* (*1946*)); others even suspect that folic acid might stimulate the production of such neurologic lesions (R. W. Heinle and A. D. Welch, *J. Am. Med. Assn.* **133,** *739* (*1947*)).

Nontropical sprue is another disease for which early reports of dramatic and successful treatment with folic acid were frequently recorded (W. J. Darby and E. Jones, *Proc. Soc. Exp. Biol. Med.* **60,** *259* (*1945*); C. V. Moore, O. S. Bierbaum, A. D. Welch, and L. D. Wright, *J. Lab. Clin. Med.* **30,** *1056* (*1945*)); similar and even more dramatic results were noted in the treatment of tropical sprue with folic acid (T. D. Spies *et al.*, *South. Med. J.* **39,** *30* (*1946*)). These encouraging reports also have been challenged (J. F. Weir and M. W. Comfort, *J. Lab. Clin. Med.* **32,** *1231* (*1947*); L. S. P. Davidson, R. H. Girdwood, and E. M. Innes, *Lancet* **I,** *511* (*1947*)).

Weir and Comfort have reported the results of treatment in 7 cases of nontropical sprue. Their plan of study was essentially that of careful observation and comparison of the condition of their patients before, during, and after treatment with folic acid. In addition to detailed histories, rather complete laboratory studies were carried out; sternal aspiration was done for all patients. The fecal solids, fat, and nitrogen of 4 of the 7 subjects were determined each

day during a three day test period while the patients were subsisting on standard test diets containing 101.6 g. of fat, 117.5 g. of protein, and 269.6 g. of carbohydrate. On completion of a preliminary survey of this type, the patients were maintained on a diet containing 50 to 100 g. of fat, 120 g. or more of protein, and carbohydrate to raise the caloric intake from 3000 to 3500 calories. The dose of folic acid employed was 50 mg. daily administered intramuscularly. Hematologic and chemical determinations of the blood were made each week. The reticulocyte count was made every other day. Five of the 7 patients were kept under hospital observation for four weeks or longer. On dismissal from the hospital, the patients continued to take folic acid intramuscularly or orally and, in addition, most of them took calcium lactate in doses of 3.9 g. three times a day. Each subject on his return home was requested to keep a diary in which was recorded the number of stools passed daily, as well as other manifestations of the disease which might be of interest.

A detailed history and the laboratory results in the 7 cases are tabulated by the authors. Only 2 of the patients receiving folic acid showed any significant gain in weight, improvement in strength, or in a sense of well-being. One of these patients had had symptoms of nontropical sprue and diabetes mellitus for three years; diarrhea had not been present on admission, although the stools contained excess fat. Even before treatment with folic acid had been instituted, this patient had gained 10 pounds, and further gain was accomplished after this treatment was begun. It was considered that the gain in weight was due largely to increased intake of food and control of the diabetes, because the sprue was in remission at the time of admission.

On dismissal, the patient was taking 15 mg. of folic acid daily, by mouth, and progressed well for three months. Then, an exacerbation of the sprue syndrome occurred, resulting in death. A second patient showed definite improvement, but it was not possible to state the chief factor responsible for this.

In general, in the remaining 5 patients the diarrhea and steatorrhea present before treatment with folic acid persisted during treatment with this preparation, often with severe flare-ups. There was no gain in weight, strength, or sense of well-being that could not be attributed to remission of the disease and an improved intake of food. The levels of calcium and protein in the blood and manifestations of tetany or edema, when present, were not improved permanently in any case, even with an improved intake of food, folic acid, or other measures. Fecal fat and the excretion of nitrogen of 2 of these 5 subjects were measured before, during, and after folic acid therapy. Folic acid therapy had no significant effect on these values. There was some degree of anemia present in all cases at the time of admission, but the value for hemoglobin was 10 g. or more in all but one. There was no significant change in the blood counts noted during treatment with folic acid. Macrocytosis persisted after treatment with folic acid in each of the 5 cases in which re-examination was done. In general, there was little change in the reticulocyte count. In one case a megaloblastic bone marrow before treatment with folic acid became normoblastic after treatment. In 4 other cases the sternal marrow was normoblastic before treatment was started. Some of these patients had taken liver extract for varying lengths of time before their original observation at the hospital.

The authors noted that these results are in distinct contrast to the rather favorable results reported in the literature regarding the response of tropical sprue and nontropical sprue to treatment with folic acid. It is not known whether the conditions are different or whether the conditions in the cases reported had advanced to an irreversible stage and become

resistant to treatment. The relation of tropical sprue to nontropical sprue is as yet not well defined.

It is perhaps well to recall that nontropical sprue is a disease considered by most to be metabolic, and usually characterized by steatorrhea, diarrhea, loss of weight, hypocalcemia, hypoproteinemia, macrocytic anemia, and often many other changes. Not only do these manifestations vary considerably with each patient, but also the disease itself is characterized by a chronic, progressive course and often prolonged remissions. Liver extract, calcium, a diet low in fat and high in carbohydrate and protein with added vitamins have been the usual measures of treatment. Many patients so treated experience a remission, but the tendency toward spontaneous remission has always rendered interpretation of specific therapeutic agents most difficult. Such difficulties of interpretation are not uncommon in evaluating therapy for patients with peptic ulcer or multiple sclerosis, which are also diseases subject to remissions.

In any event, from this and other reports, it appears that folic acid is not a "cure-all" remedy for all patients with nontropical sprue, and the physician should be hesitant in claiming cures of this disease unless he is careful to take into consideration the normal history of the malady. The application of experimental data directly to therapy of human ills is a wide gap which usually must be crossed with great care and wide clinical experience.

VITAMINS IN RELATION TO ACUTE BURNS IN MAN

The stimulus of the war and the opportunity presented by the unfortunate Cocoanut Grove disaster in Boston led to extensive studies of the metabolic changes associated with burns. These studies have dealt mainly with protein, carbohydrate, and electrolyte metabolism and little information has been available concerning the vitamins although decreased ascorbic acid of the adrenal cortex in burned guinea pigs and rabbits and a decrease in the plasma ascorbic acid concentration in patients with extreme burns have been described (H. N. Harkins, *Physiol. Rev.* **25**, *531* (*1945*)). W. A. Andreae, V. Schenker, and J. S. L. Browne (*Federation Proceedings* **5**, *3* (*1946*)) found that patients with burns or fractures characteristically showed a pronounced retention of riboflavin during the first three to five days after injury. This was followed by a period of similar duration in which there occurred an increased loss of riboflavin in the urine.

C. C. Lund and associates (*Arch. Surgery* **55**, *557* (*1947*)) report the results of a study of ascorbic acid, thiamine, riboflavin, and N′-methylnicotinamide in patients with burns admitted to the Boston City Hospital during 1944 to 1945. The investigation is part of an extensive study, one aspect of which, that dealing with nitrogen metabolism has been reported already (F. H. L. Taylor, S. M. Levenson, C. S. Davidson, and M. A. Adams, *New Engl. J. Med.* **229**, *855* (*1943*)). The determinations of vitamin output were made in the Harvard Fatigue Laboratory using the field method described by that group (R. E. Johnson, F. Sargent, P. F. Robinson, and F. C. Consolazio, *War Medicine* **7**, *227* (*1945*)).

Data were obtained on 6 children, 4 of whom had minor burns, as well as in 15 adults of whom 11 had minor burns. A low concentration of ascorbic acid in the plasma, either with the patient fasting or after saturation tests, and low urinary excretion of ascorbic acid as well as of thiamine, riboflavin, and nicotinamide, were observed. The extent of the abnormalities paralleled the severity of the burns. Patients with

burns minor in extent and superficial in depth did not show these changes unless there was in addition some complicating factor such as pre-existing deficiency, low intake of food, or serious infection. In contrast, patients with extensive deep burns invariably showed notable abnormalities. These changes were greatest in the early period following injury but continued in some cases into the chronic stage. In this respect the upset in vitamin metabolism paralleled the upset in nitrogen metabolism following burns to which reference has already been made.

In some of the patients studied, preexisting deficiency no doubt was a factor. However, this cou d not have been the explanation in all the cases. Thus, one man who had been an alcoholic for the year previous to injury was given a daily supplement of 750 mg. of ascorbic acid daily, chiefly by the intramuscular route. Nevertheless, his plasma ascorbic acid concentration fell to zero in the second week and remained at this level even after 4 g. of ascorbic acid had been given in two days. Judging by the study of J. H. Crandon, C. C. Lund, and D. B. Dill (*New Engl. J. Med.* **223**, *353 (1940)*) a much more rapid rise in plasma ascorbic acid would have been expected after actual scurvy had been treated with comparable amounts of ascorbic acid.

Inadequate dosage could not have been the explanation for the abnormalities observed since the patients with the most pronounced changes actually received extremely high amounts of vitamins, far above the so-called optimum amount for healthy persons. Likewise failure of absorption could not have been a factor since in the most severely burned patients, those who showed the most striking changes, the vitamins were given slowly by intravenous or intramuscular routes. The studies of F. Sargent, P. Robinson, and R. E. Johnson (*J. Biol. Chem.* **153**, *285 (1944)*) have shown that significant quantities of the vitamins in question are not lost by excretion in sweat, even under conditions of extreme sweating. Excretion into the edema fluid or into the exudate from the burned surfaces also could not readily explain the findings since during the first week the external exudation from deep burns is minimal and it was the patients with deep burns who demonstrated the most appreciable changes. Such loss in the exudate, however, could possibly account in part for the findings in the later stages of the injury. No data were obtained to suggest an early storage of the vitamins and there was nothing to indicate whether they were excreted in forms other than those measured. Neither could any statement be made as to the possibility that the vitamins were destroyed in the burned patients without any useful function being performed by them. Considering all these possible explanations, therefore, it would seem, by exclusion, that the most likely explanation for the changes which Lund and his associates observed would be that the trauma induced by the burns and the repair required thereafter results in an increased demand for ascorbic acid and riboflavin and possibly for other vitamins.

The investigators conclude that their data indicate as a first approximation that large doses of ascorbic acid, thiamine, riboflavin, and nicotinamide are needed by severely burned patients and that some supplementation at a lower level is needed for many patients with burns of moderate extent. They suggest that 1 to 2 g. of ascorbic acid, 10 to 20 mg. each of thiamine and riboflavin, and 150 to 250 mg. of niacin be given daily to severely burned patients and that the doses may be needed for long periods. They stress at the same time that these vitamins alone will not satisfy the nutritional needs of the patient which include a high protein, high carbohydrate diet, and ample quantities of yeast, crude liver extract (given oral'y), and vitamins A and D.

DIETARY FACTORS AND LACTATION IN MICE

Evidence for the existence of one or more dietary factors necessary for lactation in rats fed synthetic rations has been previously outlined in this journal (*Nutrition Reviews* **5**, *221* (*1947*)). Synthetic folic acid (pteroylglutamic acid) was shown to produce a partial response (L. R. Cerecedo and L. J. Vinson, *Arch. Biochem.* **5**, *469* (*1944*); and *Nutrition Reviews* **5**, *311* (*1947*)), but one or more unknown factors present in a crude liver eluate powder was necessary for maximal effects (*Ibid.* **5**, *311* (*1947*)). The purified ration with which these latter results were obtained contained no added biotin.

Similar studies on factors required for reproduction and lactation in mice have been the subject of two short reports by L. R. Cerecedo and L. Mirone (*Arch. Biochem.* **12**, *154* (*1947*); **15**, *324* (*1947*)). On a purified ration containing casein, salts, sucrose, lard, and synthetic vitamins, but no folic acid, biotin, or niacin, it was first shown that supplements of synthetic folic acid greatly improved lactation performance. Of 29 litters averaging 5.3 young each, only 11 litters, containing on the average 1.8 surviving animals, were weaned. In the group receiving folic acid supplementation, the corresponding figures were: initial litters, 29 with 5.8 animals per litter; litters weaned, 23, with 4.0 animals surviving per litter.

The effectiveness of supplementation of the basal ration with xanthopterin, biotin, folic acid, or biotin plus folic acid was then compared. The three figures listed below for each group refer in order to (1) the number of animals bred, (2) the per cent of these animals carrying through successful gestation, and (3) the per cent of these litters successfully weaned. Basal (purified) ration: 56, 57, 41; basal ration plus folic acid: 127, 90, 68; basal ration plus biotin, 9, 89, 75; basal ration plus xanthopterin, 24, 50, 83; basal ration plus folic acid and biotin, 36, 97, 83; stock (commercial) ration: 82, 96, 95.

From these figures it appears that both biotin and folic acid individually enhance reproductive and lactation performance on this ration; when these two vitamins are administered together, the effectiveness of the ration approaches that of the crude stock diet. Xanthopterin alone greatly improves lactation performance, but not reproductive performance. The general conclusions concerning the beneficial effects of these supplements are confirmed by an examination of the data given in the original reports concerning the size of litters born, the size of the litters weaned, and the average weaning weights.

The effects noted above for xanthopterin are most interesting in view of the repeated assertions that this substance has ill defined physiologic effects of its own, often partially duplicating those of folic acid and its occurrence in nature (*Nutrition Reviews* **4**, *270* (*1946*)). The beneficial effects of biotin on lactation in mice have been reported independently and in greater detail by P. F. Fenton and G. R. Cowgill (*J. Nutrition* **33**, *703* (*1947*)). It will be interesting to see if combined supplementation of synthetic rations with these three substances will permit the same excellent reproduction and lactation in mice which can be obtained on natural rations, and whether biotin and xanthopterin are effective in improving the lactation performance of rats receiving folic acid.

ALPHA-TOCOPHEROL AND PROTEIN UTILIZATION

In 1946 E. L. Hove (*Proc. Soc. Exp. Biol. Med.* **63**, *508* (*1946*)) showed that administration of alpha-tocopherol reduced weight loss in rats maintained on a low pro-

tein diet (5 per cent crude casein) for long periods of time. This result appeared at the time to be in contradiction to earlier results of H. Dam (*Ibid.* **55**, *55* (*1944*)), who observed no effect of tocopherol administration on body weights of rats maintained until death on similar low protein diets supplying about 5 per cent of yeast protein.

The possible effects of alpha-tocopherol on protein utilization have now been further examined by E. L. Hove and P. L. Harris (*J. Nutrition* **34**, *571* (*1947*)). Litter mate pairs of male albino rats were placed on test diets at weaning. One member of each pair received daily the test diet plus 2 drops of olive oil; the other received the test diet plus 1 mg. of alpha-tocopherol dissolved in 2 drops of olive oil. Five to 15 animals were used in each experimental group. Rations were fed ad libitum in most experiments; in some, the equalized feeding technic was used. Careful records of food consumption were kept for four weeks. Protein efficiency ratios were calculated by dividing the four week body weight gain by the total four week protein intake. All experimental results were carefully checked statistically for significance.

Purified rations containing salts, sucrose, lard, protein source, and synthetic vitamins were fed. Protein additions were made at the expense of sucrose. Rations containing from 5 to 40 per cent of casein were fed. At the 5 and 10 per cent casein levels, groups both with and without extra cystine (0.5 per cent) were included. On diets containing 8 and 10 per cent of casein, tocopherol supplementation significantly increased growth and protein efficiency ratios over those obtained with the control groups. No significant benefits were produced by tocopherol supplementation of rations containing 6 per cent or less, or 12 per cent or more, of casein. Failure to observe beneficial results of tocopherol in rations containing only 5 per cent of casein (or 5 per cent of casein plus 0.5 per cent cystine) may have been due to the limited time (four weeks) of the experiment. As noted already, previous experiments over a twenty week period (Hove, *Proc. Soc. Exp. Biol. Med., loc. cit.*) showed marked growth and survival benefits from tocopherol administration at this level of protein.

In additional experiments with diets containing 10 or 15 per cent of yeast protein, no favorable effects of tocopherol administration were found. Furthermore, addition of small amounts of yeast protein (2 per cent to an 8 per cent casein ration) duplicated the effect of vitamin E supplementation in increasing the protein efficiency ratio. This unexplained effect of yeast protein clarifies the apparent discrepancy between the results of Hove and Dam, referred to earlier. Dam used rations containing yeast protein, and hence observed no beneficial effects of vitamin E.

K. Schwarz (*Z. f. physiol. Chem.* **281**, *109* (*1944*)) had reported that alpha-tocopherol delayed death and prevented liver damage in rats receiving diets containing alkali-washed casein as the source of protein. This effect of tocopherol, however, was duplicated by addition of the purine base xanthine, to the ration. For this reason, Hove and Harris investigated the effect of supplementing a 10 per cent casein diet with 0.1 per cent of xanthine. The results showed that this substance, too, like yeast protein, duplicated the effect of tocopherol in increasing the protein efficiency ratio. The mechanism by which two such chemically different substances as alpha-tocopherol and xanthine can produce the same effect is not known. The authors point out, however, that xanthine (K. V. Giri and P. S. Rao, *Proc. Indian Acad. Sci.* **24B**, *264* (*1946*)) functions as an antioxidant in some metal-catalyzed oxidations, and that both it and alpha-tocopherol may function nonspecifically in this capacity in the present instance.

In certain of the experiments extended over a twelve week period alpha-tocopherol

reduced the incidence of stomach ulcers and the bleaching of the maxillary incisors which occur in rats on the low protein diets. These effects were as marked, or even more so, on the 5 per cent casein diet as on the diets containing 10 per cent of casein. Similar effects of tocopherol on other rations have been discussed previously (*Nutrition Reviews* **5**, *308 (1947)*).

This investigation adds to a growing list of unexplained relation of alpha-tocopherol to nutrition. It further emphasizes how similar over-all nutritive results may be produced by diverse means.

RELIABILITY OF NUTRIENT CALCULATIONS FROM FOOD TABLES

The description and evaluation of the diets of even small population groups are never easy because of variations in food likes and dislikes, in cooking procedures, in economic circumstances, and in cultural backgrounds. To aid in the evaluation of diets of a wide variety of types, many foods have been analyzed for the content of various nutrients by a variety of methods and investigators. To make these scattered data available in convenient form, compilations such as the "Tables of Food Composition in Terms of Eleven Nutrients" (*U. S. D. A. Misc. Pub.* **572** *(1945)*) have been prepared. Always questions may be raised as to whether the average values presented in such a compilation may be applied in a given survey or whether the actual values of the foodstuffs as produced and cooked in this particular community differ to a significant degree from these average values.

In an investigation to test the validity of food values determined by calculation of dietary records, M. M. Kaser and coworkers (*Am. J. Hygiene* **46**, *297 (1947)*) made a comparison of the values for calories, vitamin A, ascorbic acid, thiamine, riboflavin, and niacin as calculated from two sets of food tables and as determined by actual laboratory analysis in eighty typical daily diet records collected during nutrition surveys of a rural population in middle Tennessee. Three day dietary intake records were collected twice during the survey period from about 1100 white and Negro subjects of all ages. From these records, forty representative diets were selected from each of the spring and fall periods to allow a comparison of the effect of seasonal variation on the dietary composition. Most of the "spring" diets were collected during February, March, and April when there were few home grown vegetables available; most of the "fall" dietary records were taken during August to October when fresh vegetables were available. The recorded composition of the first day's food of each three day record was used for these comparisons. The portions of food used for the laboratory analysis were prepared as nearly as possible from the types of foodstuffs and by approximately the same cooking procedures that were customarily used in the area from which the survey data were collected.

Two different tables of food values were used in calculating the caloric and vitamin contents of these diets. The first is referred to as the "survey table" and was compiled in 1941 from sources of food composition then available. The second is referred to as the "state table" and was compiled in 1943 by the staff of the Nutrition Service of the Tennessee Department of Health from the same and additional later sources of information. Certain corrections for losses of ascorbic acid in cooking were made in the second table which resulted in substantially lower calculated values for this vitamin when the "state table" was used.

The caloric contents of a group of "spring" diets were determined in the Emerson bomb calorimeter. The values thus ob-

tained were corrected for the protein content of the diets by using the caloric equivalent of 5.7 calories per gram of protein burned in the calorimeter and food value of 4.0 calories per gram. Ascorbic acid was determined by the method of O. A. Bessey (*J. Biol. Chem.* **126**, *771* (*1938*)) with no attempt to correct for the presence of dehydroascorbic acid. After digestion in alcoholic potassium hydroxide and extraction with petroleum ether, vitamin A was determined by means of the Carr-Price reaction and carotene by direct measurement of the intensity of the colors of the extracts with the Evelyn photoelectric colorimeter (M. M. Kaser and J. A. Stekol, *J. Lab. Clin. Med.* **28**, *904* (*1943*)). When tomatoes were present in the diet, it was necessary to separate the carotene and lycopene by the procedure of G. Mackinney, S. Aronoff, and B. T. Bornstein (*Ind. Eng. Chem.* (*Anal. Ed.*) **14**, *391* (*1942*)). Thiamine was determined by the yeast fermentation procedure of A. S. Schultz, L. Atkin, and C. N. Frey (*Ibid.* **14**, *35* (*1942*)) following digestion with papain and takadiastase (V. H. Cheldelin, M. A. Eppright, E. E. Snell, and B. M. Guirard, *Univ. Texas Pub.* **4237**, *15* (*1942*)). Riboflavin assays were made by the method of M. Landy and D. M. Dicken (*J. Lab. Clin. Med.* **27**, *1086* (*1942*)) after preliminary papain and takadiastase digestion. The riboflavin content of the "spring" diets as determined by this procedure was stated by the investigators to be several times the calculated values. Since most of the diets studied were of high fat content, and since the stimulation of lactic acid production by Lactobacillus casei has been observed in the presence of fats, riboflavin was determined after hydrolysis with tenth-normal hydrochloric acid as well as by enzymatic digestion. Determinations were made both before and after ether extraction. Some ether soluble substance (or substances) was liberated in both hydrolysis and digestion but this source of error was much greater in samples which had been digested enzymatically. If fat extraction was used, no significant difference in riboflavin content was observed between the acid hydrolysates and enzymatic digests. The values reported for the "fall" diets were obtained from extracted enzymatic digests. Niacin was determined by the method of Landy and Dicken (*loc. cit.*) with the values reported having been obtained with enzymatic digests after ether extraction.

Although there were differences of several hundred calories between corresponding figures, the means of the calculated and determined values for the caloric value of ten "spring" diets were not significantly different. Comparable data were obtained with both food tables.

Marked discrepancies between determined and calculated values were observed for the various vitamins studied in this survey. These differences were greatly minimized when diets were considered in groups instead of individually. The two largest differences were observed in the vitamin A values for "fall" diets and in the thiamine levels for "spring" diets. One of the most valuable observations resulting from these comparisons was that the determined vitamin levels corresponded a great deal better at lower than at higher levels of intake. This observation is of obvious importance since the purpose of most surveys is to determine in as exact fashion as possible the incidence of borderline and deficient diets with less concern for those diets which have a generous content of the nutrients under investigation. A further observation by these investigators of special importance in some cases was that the median value was a more reliable evaluation than the average value since the average is more heavily influenced by diets with very high vitamin content which frequently occurred. The best example of this statement was in the vitamin A figures for "fall" diets: the average of the determined vitamin A analyses was 9805 I.U. while the averages

from the calculations by the survey and state tables were 21,240 and 15,699 I.U., respectively. In contrast, the median value of the vitamin A analyses was 5470 I.U. while the median values for the survey and state tables were 5250 and 4580 I.U. respectively.

The determined values for riboflavin tended to exceed those obtained by calculation from tables. Some variation was also seen in the figures for niacin as determined by analysis and as calculated from food tables. The actual ascorbic acid content of diets was much less as determined by analysis than from food charts, although the more recent state table provided more nearly comparable values since cooking losses were estimated.

In the comparison of the data on a seasonal basis, the vitamin A, carotene, and ascorbic acid values obtained by analysis were substantially higher in the "fall" diets while the thiamine and niacin values as determined analytically were higher in the "spring" diets.

These authors cautioned that dietary calculations for single one day records should be interpreted with caution but that the errors involved in the use of calculations based on dietary records could be appreciably reduced if they represented longer periods of time. Errors could also be reduced if the diets of groups of subjects were considered in terms of median and mean values, from which the data could be interpreted with greater reliability.

ORAL USE OF PROTEIN HYDROLYSATES

The current enthusiasm for protein therapy in disease, especially the use of protein hydrolysates, has given support to certain misconceptions not the least of which is that hydrolyzed protein by mouth is nutritionally superior to a similar amount of protein in food. No doubt situations occasionally arise in which protein hydrolysates are better absorbed than whole protein, but clearcut studies to substantiate this have been few.

In an effort to clarify the matter, F. Homburger (*Am. J. Med.* **3,** *430 (1947)*) and F. Homburger and N. F. Young (*Ibid.* **3,** *427 (1947)*) outlined the indications for oral protein hydrolysates and evaluated the chemical composition of ten protein hydrolysates intended for oral use. The ten hydrolysates were analyzed for total nitrogen, ammonia nitrogen, creatine and creatinine, sodium, potassium, total phosphorus, and finally amino nitrogen by five methods: nitrous acid, a copper complex method, a crude and refined formol titration, and free amino nitrogen by the gasometric ninhydrin procedure. The results were compared with such analyses as were given by the manufacturer on the label.

The total nitrogen contents agreed very well with those on the labels. The amounts of creatine and creatinine were generally low except in the hydrolysates derived from animal sources where substantial amounts were found. The sodium content of the hydrolysates varied from 0.3 g. to 21 g. per 100 g. It is obviously important that the sodium content be clearly indicated when one considers that hydrolysates may be used in such diverse conditions as adrenal insufficiency, hypertension, and congestive cardiac failure. In some instances it was thought that the mineral values were too low if the product were to be used as a large part of the diet.

The greatest discrepancies were found between the five methods used for the determination of amino nitrogen. Generally the crude formol titration gave the highest values and they corresponded closest to the labeled content. The percentage of the total nitrogen present as amino nitrogen in nine hydrolysates varied from 24 to 57,

but in one it was only 7 per cent. As the authors point out, the latter is an extremely low value for a "hydrolyzed" protein preparation.

It was suggested that the nitrous acid method, because of its reliability, be used by all manufacturers to determine the amino nitrogen content of hydrolysates and that the content so determined appear on the label. In addition, the mineral, especially sodium, content should appear.

With these facts in mind, Homburger discusses the indications for the oral use of protein hydrolysates, emphasizing that there is little reason to prefer hydrolyzed to native protein in the presence of normal gastrointestinal function. In the presence of certain gastrointestinal diseases the author has apparently found oral protein hydrolysate to be of value. Among these he lists chronic pancreatitis, postoperative resection of the head of the pancreas, ulcerative colitis, and some cases with intestinal short-circuits following surgery. Data showing the greater effectiveness of hydrolysates over whole protein in these conditions are, however, unfortunately not presented and are almost nonexistent in medical literature. A. T. Shohl (*J. Clin. Invest.* **22,** *257* (*1943*)) found better retention of nitrogen when given in the form of protein as a hydrolysate than as milk in infants with acute gastrointestinal disturbances. C. D. West, J. L. Wilson, and R. Eyles (*Am. J. Dis. Child.* **72,** *251* (*1946*)) observed better absorption of hydrolyzed than whole protein as determined by blood amino nitrogen rise in infants with deficient pancreatic function. F. W. Co Tui *et al.* (*Gastroenterology* **5,** *5* (*1945*)) recommends the therapeutic use of protein hydrolysates by mouth in the treatment of peptic ulcer. Further clinical investigations of these problems, with appropriate control studies, are badly needed, particularly in view of the wide use of oral protein hydrolysates.

Homburger rightly emphasizes the occasionally overlooked necessity for maintaining caloric intake in patients given protein supplements so that the latter will not be used for energy.

Further discussion of the use of protein hydrolysates is given in a Cornell Conference on Therapy (*Am. J. Med.* **3,** *472* (*1947*)). The oral use of protein hydrolysates in pediatrics is considered. Particularly interesting is the observation that about 50 per cent of children with eczema could be expected to show improvement using a dietary formula containing 20 per cent amino acids, carbohydrates up to 50 per cent, and fat 18 per cent. In this way a minimum quantity of allergenic materials is ingested

NUTRITION SURVEY OF VIENNESE CIVILIANS IN 1945

C. S. Davidson, H. L. Wilcke, H. D. Fein, and P. J. Reiner (*J. Lab. Clin. Med.* **32,** *1470* (*1947*)) have reported the results of a survey of the nutritional status of 2325 Viennese civilians under United States occupation in the summer of 1945. The observations were made in two areas, one of which was a representative section of the inner part of the city while the other represented the outlying districts. What seemed to be very satisfactory precautions were taken to ensure an adequate sampling of the population. The inhabitants varied from the poorest in the city to some of the most wealthy. Many professional men and women were included as well as workers, housewives, children, and disabled persons. Those studied represented close to 1 per cent of all those living in the areas.

The persons studied, especially the adults, showed a marked reduction in weight below the United States standard for height and weight. Almost half of those examined were more than 10 kg. below the U. S.

standard. The general appearance of the individuals was not that of extreme undernutrition but simply of weight loss. Many appeared normal, a few plump, but most showed evidence of considerable loss of weight, and a few showed extreme loss of weight. In the last, posture was poor, movements were carried out with a characteristic lethargy, and the facial expression was apathetic. Abnormal pigmentation was not seen. Many individuals spontaneously complained of weakness, fatigue, and even syncope.

As has been the case in other surveys, surprisingly few signs suggestive of specific vitamin deficiencies were observed. There was no evidence of beriberi, peripheral neuritis, or of pellagra and only one individual with classic scurvy was seen. Follicular hyperkeratosis, cheilosis, glossitis, abnormal tongue color, abnormal color and texture of the hands, corneal vascularity, and parasthesias, signs that have been described as suggesting specific deficiencies, were uncommon. Diffuse, nontoxic goiter was present in 21 to 42 per cent of the individuals. Distinct papillary atrophy of the tongue was seen in 156 cases. However, in 60 of these the mouth was edentulous so that the significance of the findings in these individuals is open to question. A beefy red or a magenta colored tongue was observed in 404 individuals of whom 102 were edentulous. The combination of papillary atrophy and either beefy red or magenta colored tongue was seen in 32 cases, 17 being edentulous. From 10 to 20 per cent of the adults observed showed symptoms suggesting acrocyanosis. In the morning, particularly if cold, the hands and feet were cold to the touch and presented a mottled cyanotic appearance. In the warmer part of the day the cyanosis might give way to a mottled reddish flushing but the hands were usually still cold to the touch. Paresthesia of the hands and feet in association with this acrocyanosis was seen in only 40 cases. Thus the correlation of the two was not as striking as had been found in starved prisoners (C. S. Davidson, H. L. Wilcke, and P. J. Reiner, *J. Lab. Clin. Med.* **31,** *721 (1946)*).

The majority of the serum protein determinations were either normal or slightly reduced and hence the averages were only slightly below normal. Nevertheless, a significant number of individuals had hypoproteinemia. Twenty-eight per cent of the values were 5.5 g. per 100 ml. or below and over 50 individuals had serum protein concentrations between 4.5 and 5.0 g. per 100 ml.

Pitting edema was observed in 133 individuals. There were 45 with edema in whom no clinical cause could be found. These were considered to have "hunger edema." The average weight loss of the persons with hunger edema was definitely more than in those with dependent edema for which a cause was discovered. Anemia, as measured by hemoglobin determined by the specific gravity method, was present though mild. A relatively small proportion of the individuals had hemoglobin concentrations of 15.5 g. per 100 ml. or over. Absent menses of two months' duration or more, not due to pregnancy, were present in 16 per cent of the women between the ages of 15 and 44. Analysis of the average food consumption at the time of the survey showed an intake of from 1000 to 1440 calories with about 50 g. of protein per day.

For the low incidence of signs associated with specific vitamin deficiencies, a finding consistent with that of most of the other reports from Europe, Davidson and associates suggest three possible factors. The duration of the poor diet may not have been long enough to permit the development of signs of deficiency; the food did contain some of the vitamin B-complex even though inadequate amounts; and, finally, the metabolic rate was probably significantly re-

duced. Furthermore, the low caloric intake would have reduced the requirement for vitamins of the B-complex. No attempt is made to explain the appearance of papillary atrophy and redness of the tongue in the persons studied since these signs occurred largely in edentulous individuals. It is pointed out that incidence of these signs does not necessarily signify a deficiency in vitamin B-complex.

URINARY EXCRETION OF PTEROYLGLUTAMIC ACID AND CERTAIN RELATED COMPOUNDS

The next advance in the understanding of the pathogenesis of pernicious anemia and related disorders would seem to depend on a better understanding of the role of pteroylglutamic acid in metabolism. Consequently, studies dealing with the urinary excretion of this substance and of related compounds are of importance.

In the early stages of the investigations of Lactobacillus casei factor (folic acid) it became evident that only small amounts were excreted in the urine of normal human subjects (L. D. Wright and A. D. Welch, *Science* **98,** *179* (*1943*)). The amount excreted daily was estimated to be less than 1 per cent of the probable dietary intake. It was suggested that dietary pteroylglutamic acid was probably broken down before excretion. When pure pteroylglutamic acid became available for administration as a supplement, it was shown that the amount of this substance excreted in the urine increased sharply as the dosage was raised, indicating that the capacity of the body to break down pteroylglutamic acid is limited.

C. W. Denko and associates (*Arch. Biochem.* **10,** *33* (*1946*)) found that the average daily urinary folic acid excretion in young men on a normal diet was about 4 micrograms as measured by assay with Streptococcus faecalis R. Supplementation with a very small amount (90 micrograms) of pteroylglutamic acid daily did not increase the urinary excretion (Denko *et al.*, *Ibid.* **11,** *109* (*1946*)). R. M. Suárez *et al.* (*J. Lab. Clin. Med.* **31,** *1294* (*1946*)) likewise found the daily urinary excretion of pteroylglutamic acid to be 2 to 4 micrograms, rarely exceeding 5 micrograms, on ordinary diets. Following oral or parenteral administration of this substance, 15 to 75 per cent of the amount administered was excreted, within twenty-four hours, depending on the size of the dose. R. Steinkamp, C. F. Shukers, J. R. Totter, and P. L. Day (*Proc. Soc. Exp. Biol. Med.* **63,** *556* (*1946*)) observed that when doses of 5 to 16 mg. of pteroylglutamic acid were fed to normal subjects an average of 28.5 per cent of the administered dose was excreted. Most of the excretion took place between the second and eighth hour after dosage. Hospital patients excreted much lower percentages of the administered dose. M. E. Swendseid, O. D. Bird, R. A. Brown, and F. H. Bethell (*J. Lab. Clin. Med.* **32,** *23* (*1947*)) found in normal subjects that only about 3 micrograms of pteroylglutamic acid were present in the twenty-four hour urine prior to administration of this vitamin whereas when 4 mg. of the compound were given, about 32 per cent of the dose was excreted in the urine in twenty-four hours.

T. H. Jukes, A. L. Franklin, E. L. R. Stokstad, and J. W. Boehne (*J. Lab. Clin. Med.* **32,** *1350* (*1947*)) describe further observations on normal adult men. In their first series the urinary excretion of pteroylglutamic acid was studied following administration of various dosage levels of a solution of the sodium salt. The results showed clearly that the proportion which was excreted increased as the dose was

raised. Furthermore, the greater part of the excretion took place in the first six hours. A small part of the administered dose was excreted in the next eighteen hours. An average of 53 per cent of a dose of 5 mg. was excreted in twenty-four hours and an average of 90 per cent of this excretion took place in the first six hours. After twenty-four hours the urinary folic acid content returned to the basal level.

Following administration of pteroyltriglutamic acid the urinary excretion values were similar to those found with pteroylglutamic acid. Thus it appears that pteroyltriglutamic acid is readily hydrolyzed in the body to pteroylglutamic acid. There was some indication of a lag in excretion, the proportion of the biologic activity excreted in the six to twenty-four hour period being greater with pteroyltriglutamic acid than with pteroylglutamic acid. It was of interest that the excretion rate appeared to be the same following dosage by either the oral or intravenous route, thus suggesting that the breakdown of pteroyltriglutamic acid to pteroylglutamic acid can take place in the body with equal rapidity whether or or not the triglutamic acid compound passes into the body through the gastrointestinal tract.

The triglutamic acid compound has about 3 per cent of the activity of pteroylglutamic acid in S. faecalis R assay. Pteroyldiglutamic acid has practically no activity in stimulating the growth of these organisms. Urinary excretion studies indicated, nevertheless, that the body possesses the ability to liberate pteroylglutamic acid from pteroyldiglutamic acid. However, even more than in the case of pteroyltriglutamic acid there was a lag in the rate of excretion so that a comparatively large but variable proportion was found in the urine during the period between six and twenty-four hours after dosage. The effect was especially marked following intravenous administration.

When the diamide of pteroylglutamic acid was studied it was found that no increase in the urinary excretion of pteroylglutamic acid occurred in the twenty-four hour urine samples.

The apparent ability of the body to hydrolyze pteroyldiglutamic acid as evidenced by these urinary excretion data is in contrast to the microbiologic activity of this substance but may be in harmony with its reported effect in producing a remission in macrocytic anemia (T. D. Spies, R. E. Stone, and R. L. Toca, *South Med. J.* **40,** *175 (1947)*). Jukes and associates point out that the inactivity of pteroyldiglutamic acid for L. casei and S. faecalis R may indicate that these organisms do not possess a conjugase system which is effective in hydrolyzing the peptide linkage in pteroyldiglutamic acid between the terminal glutamic acid and the alpha-carboxyl group of the glutamic acid radical of the pteroylglutamic acid moiety of the molecule. In contrast pteroyltriglutamic acid is fully active for L. casei which may mean that this organism has a conjugase system which hydrolyzes the gamma linkage between glutamyl groups. An alternative explanation, they point out, would be that L. casei utilizes both pteroylglutamic and pteroyltriglutamic but not pteroyldiglutamic acid as precursors of more complex substances.

The site of the breakdown of the pteroylglutamic acid compounds is not known but preparations made from various organs have been shown to have vitamin Bc conjugase activity (O. D. Bird *et al., J. Biol. Chem.* **159,** *631 (1945)*) as indicated by liberation of pteroylglutamic acid from pteroylheptaglutamic acid.

NOTES

Keratins for Poultry Feeding

Keratins such as powdered hog hoofs, wool, and chicken feathers are normally waste products in the animal industry, yet these may have considerable nutritive value. Earlier studies (see *Nutrition Reviews* **2,** *75* (*1944*)) demonstrated that when rat or chick diets contained powdered hoofs at a 30 per cent level, as good growth was obtained as at an 18 per cent level of casein. Such a waste product might therefore replace considerable amounts of grain, meat scraps, or fish meal in a practical poultry ration.

Further studies (G. W. Newell and C. A. Elvehjem, *J. Nutrition* **33,** *673* (*1947*)) have generally confirmed the value of powdered hoof keratin as a protein source. A level of 18 per cent powdered hoofs and 10 per cent casein was nearly as favorable in growth response of the chick as 18 per cent casein, 10 per cent cartilage. Good feathering of the chicks was observed at all levels of powdered hoof tested. The method of powdering the sample influenced the value of the product, and heating of the preparation was found detrimental.

Powdered hog hair, even in the presence of considerable casein, led to an undesirable incidence of encephalomalacia, and powdered chick feathers never led to satisfactory growth and feathering.

The keratins are deficient in tryptophane, methionine, histidine, and lysine, and the addition of these amino acids enhances considerably the value of the keratins. Casein and perhaps other proteins satisfactorily supplement the deficiency. The use of powdered keratins in poultry feeds, with adequate supplementation, might contribute indirectly but significantly to increased availability of food for man.

Correction

In the review entitled "Nutritive Value of Casein and Lactalbumin for Man" (*Nutrition Reviews* **6,** *61* (*1948*)) it is stated that comparative studies of the nutritive value of casein and of lactalbumin were made on dogs by H. B. Lewis, on mice by C. D. Bauer and C. P. Berg, and on chicks by H. J. Almquist. Dr. Lewis has called attention to the fact that such studies were not reported in the cited reference to his work. The same criticism applies to all three references. The Editor regrets the repetition in *Nutrition Reviews* of errors in a paper used in the preparation of the above review.

Protein Consumption and Blood Urea and Creatinine Concentrations in Man

Of interest to nutritionists is a series of papers concerned with the renal excretion and clearance of urea and creatinine.

In the first paper (T. Addis, E. Barrett, L. J. Poo, and D. W. Yuen, *J. Clin. Invest.* **26,** *869* (*1947*)) the blood urea concentration was determined before each meal and before retiring for each of three levels of protein consumed for six days by 10 normal individuals. The food protein levels chosen were 0.5, 1.5, and 2.5 g. per kilogram of body weight. The serum urea determinations averaged 19.3 mg. per 100 ml. at the 0.5 g. protein intake level, 38.6 for 1.5 g. protein, and 45.5 at the 2.5 g. level.

In the second paper (Barrett and Addis, *J. Clin. Invest.* **26,** *875* (*1947*)) measurements of the serum creatinine are reported which were made on the same subjects at the same time as the serum urea concentrations. In contrast to the latter, however, variations in protein consumption had no measurable effect upon the serum creatinine which was, in fact, very constant, averaging 1.020 mg. per 100 ml.

The third paper (Addis, Barrett, and J. T. Menzies, *J. Clin. Invest.* **26,** *879* (*1947*)) describes a simple clinical method for determining the approximate serum creatinine concentration. The method is compared with the standard Folin-Wu method and although errors in the simple method occur,

they were not great enough to detract from its clinical usefulness.

Vitamin D and Feather Pigmentation

Little is known of the mechanism by which vitamin D exerts its physiologic effects. Observation of new results of vitamin D deficiency in experimental animals are therefore of considerable interest. E. W. Glazener, J. P. Mattingly, and G. M. Briggs (*Poultry Sci.* **25,** *85 (1946)*) reported that the amount of black pigment in the feathers of New Hampshire chicks fed vitamin D deficient diets was increased. The New Hampshire chicks normally contain some black pigment in the feathers.

A. Decker and J. McGinnis (*Proc. Soc. Exp. Biol. Med.* **66,** *224 (1947)*) note the same phenomenon in Buff Orpington chicks on vitamin D deficient rations. In this breed, occurrence of the black pigment is especially striking, since the normaily buff feathers contain none of it. The degree and distribution of the black pigment, which becomes most noticeable at 4 to 6 weeks of age, varies considerably in the various deficient chicks. Under favorable conditions a distinct band of black pigment can be discerned. When such birds are supplemented with vitamin D, feathers of normal color are produced. Nothing is yet known of the mechanism by which this effect is produced.

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The publication of NUTRITION REVIEWS has been undertaken to enable professionally trained people to keep abreast of current progress, and to have available an unbiased, authoritative review of the current research literature in the science of nutrition.

The Editorial Staff has been carefully selected so that the publication will merit in reasonable degree, the confidence of the medical profession and others who work directly with the public in the field of nutrition. The service provided by the publication is distinct from that of an abstract journal, a review for laymen, or an extensive scientific review. Since the reviews as originally prepared by a member of the Editorial Staff are frequently modified by special referees and may be further modified by the Editorial Office in preparing final copy for the press, the Editorial Committee feels that it is preferable not to affix the names of Editorial Staff members to individual reviews. There is thus a high degree of group responsibility on the part of the Editor and Assistant Editors.

Signed articles do not necessarily represent the opinion of the Editorial Staff.

A leaflet based on articles appearing currently in NUTRITION REVIEWS and entitled "Current Research in the Science of Nutrition" is distributed free of charge by the Nutrition Foundation each month. The vocabulary of the leaflet is adapted for lay reading and the subject matter is restricted to clearly established findings of practical interest. Those who wish to have their names placed on the mailing list should write directly to the Nutrition Foundation, Inc., Chrysler Building, New York City.